CLAIMS

(1) A method for evaluating RNAi activity, comprising the steps of:

supplying a target-expressing molecule, which is a polynucleotide containing at least a target sequence and an expression regulatory region which regulates an expression of an RNA containing said target sequence, and

a subject nucleic acid molecule to be evaluated on whether or not the subject nucleic acid molecule has an RNAi activity on said RNA containing the target sequence

into an expression system in which said target-expressing molecule is capable of expressing said RNA containing the target sequence; and

detecting whether or not said RNA containing said target sequence has been cleaved.

(2) The evaluation method for RNAi activity of claim 1, wherein the target-expressing molecule comprises, both upstream and downstream of the target sequence, a PCR primer annealing region containing a sequence to which a PCR primer can anneal, said method comprising the steps of:

quantifying said RNA containing the target sequence generated by supplying said target-expressing molecule and said subject nucleic acid molecule into said expression system by RT-PCR using a pair of primers annealing to the sequence in the PCR primer annealing region; and

detecting whether or not said RNA containing the target sequence has been cleaved by comparing with the case in which said subject nucleic acid molecule is not supplied into said expression system.

(3) The method for evaluating RNAi activity of claim 2, wherein an intron is flanked within at least one of said PCR primer annealing regions present both upstream and downstream of the target sequence, and said expression system is an expression system capable of splicing an RNA.

(4) The method for evaluating RNAi activity of claim 1, wherein said target-expressing molecule comprises a sequence encoding an expression product detectable as a reporter at a location whereby said reporter is to be transcribed as an mRNA integrated with the target sequence, the method comprising a step of detecting the expression of said reporter to detect whether or not the RNA has been cleaved.

(5) The method for evaluating RNAi activity of any one of claims 1 to 4, wherein the expression system is a cell-free expression system or a cell expression system.

(6) The method for evaluating RNAi activity of any one of claims 1 to 5, comprising a step of supplying into said expression system into which said target-expressing molecule and said subject nucleic acid molecule are supplied a control-supplying molecule that comprising a control sequence which comprises a sequence to be transcribed into an mRNA that is not cleaved by said subject nucleic acid molecule and encodes a detectable expression product.

(7) The method for evaluating RNAi activity of any one of claims 1 to 5, wherein a control sequence which comprises a sequence to be transcribed into an mRNA that is not cleaved by said subject nucleic acid molecule and encodes a detectable expression product is integrated into said target-expressing molecule.

(8) The method for evaluating RNAi activity of any one of claims 1 to 7, wherein said target sequence comprises a nucleotide sequence different from the nucleotide sequence that said subject nucleic acid molecule comprises.

(9) A method for evaluating miRNA activity, comprising the steps of:

supplying a target-expressing molecule, which is a polynucleotide comprising:

at least a target sequence, an expression regulatory region regulating an expression of an RNA containing said target sequence, and a sequence encoding an expression product detectable as a reporter

at a location whereby said reporter is to be transcribed as an mRNA integrated with said target sequence; and

a subject nucleic acid molecule to be evaluated on whether or not said subject nucleic acid molecule has an RNAi activity on an RNA containing said target sequence

into an expression system in which said target-expressing molecule is capable of expressing said RNA containing the target sequence; and

detecting whether or not said RNA containing the target sequence has been cleaved.

(10) The method for evaluating miRNA activity of claim 9, wherein said expression system is a cell-free expression system or a cell expression system.

(11) The method for evaluating miRNA activity of claim 9 or 10, comprising a step of supplying into said expression system into which said target-expressing molecule and said subject nucleic acid molecule are supplied a control-supplying molecule which comprises a control sequence comprising a sequence whose expression is not suppressed by said subject nucleic acid molecule and encodes a detectable expression product.

(12) The method for evaluating miRNA activity of claim 11, wherein said control-supplying molecule is integrated into said target-expressing molecule.

(13) The method for evaluating miRNA activity of any one of claims 9 to 12, wherein said target sequence comprises a nucleotide sequenced different from the nucleotide sequence that said subject nucleic acid molecule comprises.